Annals of Clinical and Analytical Medicine
Original Research

IL-10, IL-13, IL-17 and CXCL8, CXCL10: Their relationship with the development and prognosis of type 1 and type 2 diabetes

Cytokines and diabetes

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Abstract

Aim: Diabetes mellitus (DM) is a chronic high blood sugar disorder that can be classified into two groups: type 1 diabetes (T1D) and type 2 diabetes (T2D). The equilibrium between cytokines and chemokines is important for the development and prognosis of the disease. This study aimed to examine the contribution of pro-inflammatory (IL-17, CXCL8, CXCL10) and anti-inflammatory (IL-10, IL-13) factors to the development and prognosis of T1D and T2D.

Material and Methods: Cytokine and chemokine levels were evaluated in serum samples of patients with T1D and T2D, and healthy controls. Patients were divided into two groups depending on the history of the disease, as 1-5 years and 5-10 years. HbA1C levels were assessed by HPLC. Concentrations of IL-17, CXCL-8, CXCL-10 and IL-10, IL-13 were analyzed using LUMINEX.

Results: A statistically significant difference was observed in the HbA1c levels of all patients versus controls. CXCL8 was increased in patients with T1D compared with the controls. There was a significant difference in IL-13 levels between patients with T1D or T2D over five years and controls. For IL-10 and CXCL-10, there was a significant difference between T1D for more than 5 years and T1D for 5 years or less.

Discussion: Cytokines and chemokines are belonging to the immune system, but it is now known that they may also affect non-immune mechanisms. As a result, it is advantageous to take these factors almost equally into account in the diagnosis and treatment of T1D and T2D.

Keywords

Cytokines, Chemokines, Diabetes

DOI: 10.4328/ACAM.21655 Received: 2023-02-15 Accepted: 2023-03-27 Published Online: 2023-04-19 Printed: 2023-06-01 Ann Clin Anal Med 2023;14(6):541-545
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This study was approved by the Clinical Research Ethics Committee of Istanbul University Faculty of Medicine (Date: 2016-08-08, No: 930)

Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia caused by a deficit of insulin secretion and/or effect. Diabetes is divided into two primary groups as type 1 diabetes (T1D) and type 2 diabetes (T2D).

T1D is an autoimmune disease that develops depending on the selective destruction of pancreatic beta cells because of underlying genetic factors. T2D is characterized by peripheral insulin resistance, dysregulation of liver sugar production, and beta cell dysfunction. Recent studies have been carried out on the relationship between DM and insulin resistance and the immune system. B and T-cells have been reported to play a damaging and protective role in insulin resistance. Islet endocrine cells are known to produce certain cytokines that have pro-inflammatory or anti-inflammatory effects. Studies have shown that disturbance of the equilibrium between pro-and anti-inflammatory cytokines may result from the development of T1D or T2D. In both diseases, islet cells develop a complex inflammatory environment, and the microenvironment determines the fate of the islet.

Interleukin-17 (IL-17, also known as IL-17A) is a pro-inflammatory cytokine that relates the activation of T cells to the mobilization and activation of neutrophils. The activation of the IL-17 pathway in peripheral tissues and islet cells characterizes human autoimmune diabetes and promotes cell death induced by cytokines [1]. Clinical data suggests that IL-17-producing cells contribute to the underlying pathology in the early stages of the disease, and therapeutic approaches aimed at Th17-cell inhibition could be a substantial contribution to T1D treatment [2]. In the case of T2D, IL-17 has recently been shown to play a crucial role in systemic inflammation, insulin resistance, T2D and related complications [3].

Interleukin-10 (IL-10) is an anti-inflammatory cytokine, which has a suppressive effect on host defense mechanisms. It was observed that IL-10 has lower circulation rates in patients with T2D, while circulation rates are higher in patients with T1D [4]. In another study, it was found that when IL-10 shows increased genetic expression in T2D patients, metabolic control is achieved [5].

IL-13 was demonstrated to influence beta-cell survival with the protection of beta-cells from IL-1ß-triggered apoptosis [6]. The role of IL-13 in the development of insulin resistance and T2D has not been entirely clarified, while peripheral blood mononuclear cells (PBMC) in patients with T2D exhibit decreased proliferative responses [7]. One of the most important similarities between T1D and T2D is that the soluble factors released by beta cells work like chemotactic cytokines (chemokines).

CXCL8 (interleukin-8) and CXCL10 are proinflammatory chemokines. CXCL8 belongs to the chemokine family, which acts on the CXCR1 and CXCR2 receptors [8]. CXCL8 is secreted from monocytes and activates macrophage and endothelial cells [8]. CXCL8 is also involved in the migration of neutrophils to the site of inflammation and increases their phagocytic activity.

CXCL10 inhibits the formation of neovascularization and acts as an angiostatic factor [9]. CXCL10 binds to CXCL3 and regulates immune responses through the recruitment and activation of T, eosinophilic, monocyte and NK cells [9]. A recent study has demonstrated altered expressions of CCL5 and CXCL1 patients with T1D [10]. Expression of CXCL-10 is greater in isolated islets in individuals with T1D or T2D than in healthy controls [11]. In light of this background, we aimed to determine serum IL-10, IL-13, IL-17, CXCL-8 and CXCL-10 cytokine and chemokine levels to observe the development of the disease and its effects on prognosis and to obtain clues for treatment. In this context, we tried to determine serum IL-10, IL-13, IL-17, CXCL-8 and CXCL-10 levels of cytokines and chemokines to observe the progress of the disease and its effects on the prognosis and to get clues for treatment.

Material and Methods

Thirty patients with T1D or T2D without any malignancy were included in the study. The patients were referred to the study by the Diabetes Outpatient Clinic, Department of Internal Medicine, Division of Endocrinology and Metabolic Diseases, Istanbul Faculty of Medicine, Istanbul University. Thirty healthy individuals of similar age, who had not previously been diagnosed with any disorders and did not use any medications, were included in the control group.

This study was approved by the Clinical Research Ethics Committee, Faculty of Medicine, Istanbul University on 08.08.2016 with issue 930. Patients and healthy controls who agreed to take part in the study were informed about the study and signed the informed consent form.

Venous blood samples from patients and healthy controls were collected into 10 mL gel blood collection tubes. Blood samples were immediately transported by cold chain to the laboratory of Istanbul University Aziz Sancar Institute of Experimental Medicine, Department of Immunology. Serum was separated by density gradient centrifugation and stored at -80°C.

Collection of the demographic data

Patients were divided into four groups according to the etiopathogenic type of their diabetes and also according to the duration of their illness: 1. Patients suffering from T1D for more than 5 years (T1D > 5 years), 2. Patients suffering from T1D for 5 years or less (T1D ≤ 5 years), 3. Patients suffering from T2D for more than 5 years (T2D > 5 years), 4. Patients living with T2D for 5 years or less (T2D ≤ 5 years) 5. Healthy Controls.

Determination of HbA1c levels

All HbA1c measurements were performed in patients' venous blood by High Performance Liquid Chromatography (HPLC) using the Premier Hb9210 HbA1c Analyzer (Trinity Biotech, Ireland). The normal value of HbA1c is 3-6%.

Measurement of cytokine and chemokine levels

The frozen serum samples were thawed in an incubator at 37ºC. IL-10, IL-13, IL-17, CXCL8 and CXCL10 levels were measured with the "Human Magnetic Luminex Screening Assay (LXSAHM; R&D Systems, Inc., Minneapolis, MN, USA) kit" and the steps specified in the manufacturer's protocols were followed. The detection thresholds for these mediators are as follows: CXCL8 (1.8-1255 pg/mL), CXCL10 (1.18-690 pg/mL), IL-10 (1.6-1162 pg/mL), IL-13 (32.4-110.52 pg/mL), IL-17A (1.8-3.110 pg/mL).

Statistical evaluation

Distribution patterns in the study groups were assessed using the Kolmogorov-Smirnov test. The study groups with normal distribution were compared by "ANOVA method". The "TUKEY

HSD" test was used to perform post-hoc comparisons. "Non-parametric Kruskal-Wallis one-way ANOVA" was used to evaluate groups that did not show normal distribution, and "Dunn's Test" was used for post hoc comparisons. P<0.05 was accepted as the significance level. Data were represented using box plots to show the median, 25th, 50th and 75th percentiles and endpoint values. SPSS 23 software was used for all statistical evaluations and graphs.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

Table 1 presents the demographics of the patient group, as described in the materials and methods, demographic data collection, and healthy controls.

A statistically significant difference was observed in the HbA1c levels of all patients compared to controls (p<0.0001), while there was no statistically significant difference between the groups in the evaluation of IL-17.

In the comparison of CXCL10 serum levels, there was a significant difference between T1D for five years or less compared to healthy controls and patients with T1D for more than five years (p<0.0001). A statistically significant difference was observed between patients with T1D for five years or less and patients with T2D for more than five years (p<0.0001), between patients with T1D for five years or less and patients with T2D for five years or less (p<0.0001) (Figure 1-a).

When comparing serum CXCL8 levels, CXCL8 was found to be higher in patients with T1D than in controls (p<0.003). A significant difference close to healthy controls was found in patients with T2D for five years or less (p<0.002), while a lower level of significance was observed in patients with T2D for more than five years (p<0.009) (Figure 1-b).

A comparison of serum IL-13 concentrations revealed a statistically significant difference between patients with T1D for more than five years and healthy controls (p<0.001). There was a statistically significant difference in IL-13 levels between patients with T2D for more than five years and healthy controls (p<0.005). There was a statistically significant difference in IL-13 rates between patients with TD2 for five years or less and healthy controls (p<0.008) (Figure 1-c).

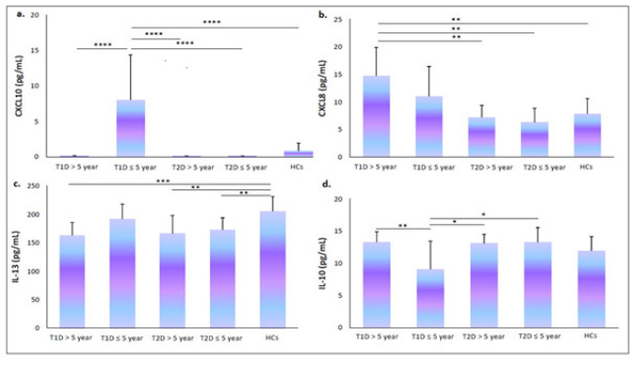

Figure 1. Bar graph demonstration of CXCL10, CXCL8, IL-13, IL-10 (pg/mL) levels among different groups depending on the type and duration of diabetes. Used to denote: "*: p<0.05", "**: p<0.01", "***: p<0.001", "****: p<0.0001".

Table 1. Demographic data of patients and healthy controls participated in the study.

	Age (year)	Sex	Disease Duration (year)	HbA1C Levels (%)	Fasting Glucose Level (mg/dL)
Type 1 Diabetes	40.89±18.91	17 Women 13 Men	8.03±5.34	8.63±1.42	183.96±54.49
Type 2 Diabetes	48.67±13.46	17 Women 13 Men	7.90±6.15	8.64±1.50	208.71±51.23
Healthy Controls	36.48±11.60	8 Women 22 Men	-	5.33±0.64	89.10±6.00

When comparing serum IL-10 concentrations, they were found to be higher in patients with T1D for more than five years compared to five years or less (p<0.01). Serum concentrations of IL-10 in patients with T2D for 5 years or less were higher than those in patients with T1D for 5 years or less (p<0.017). When the IL-10 levels of patients with T1D for five years or less were compared with T2D for more than five years, a statistically significant difference was observed (p<0.026) (Figure 1-d).

Correlation evaluation showed that in patients with type 1 diabetes, a positive correlation was found between IL-13 and CXCL8 (r=0.574), between IL-13 and HbA1c (r=0.466), between CXCL8 and patient age (r=0.555), while a negative correlation was found between CXCL8 and fasting blood glucose (r= -0.458), between IL-13 and fasting blood glucose (r= -0.335), between CXCL10 and IL-10 (r= -0.918).

In patients with type 2 diabetes, a positive correlation was found between CXCL8 and patient age (r= 0.344), between CXCL10 and CXCL8 (r= 0.320), IL-13 and HbA1c (r= 0.333), CXCL8 and HbA1c (r=0.516), IL-17A and HbA1c (r=0.622), while a negative correlation was found between fasting blood glucose and HbA1c (r= -0.359), IL-13 and IL-17A (r= -0.396), IL-17A and IL-10 (r= -0.622), IL-13 and CXCL10 (r= -0.584).

Discussion

T1D and T2D have different pathogenesis in disease development, but one of the most important commonalities between T1D and T2D is that soluble factors released from beta cells function as cytokines and chemokines that can affect prognosis [11].

The balance between the pro-inflammatory (Th1 and Th17) and anti-inflammatory (Treg) subgroups is essential for maintaining homeostasis and preventing inflammatory diseases [12]. Within T2D, the adaptive immune system regulates systemic inflammation leading to insulin resistance and related complications [13]. T1D is mediated by Th1 cells that produce IFN-gamma, but, recently, Th17-secreting IL-17 cells have also played a role [14]. Activation of peripheral pathways and islets of IL-17 and its expression promotes autoimmune response and cytokine-mediated beta cell death by inducing the synthesis of other cytokines and chemokines, including CXCL8 [14].

A study has reported increased levels of CXCL8 in T1D patients, in comparison with healthy controls as well as type 1 diabetes non-affected siblings [15]. In our study, there was no statistically significant difference between the groups in the evaluation of IL-17, while CXCL8, whose production is known to

be stimulated by IL-17, was found to be increased in the serum of T1D patients, compared to controls. In the comparison of T1D and T2D, a significant difference was found in patients with T2D for five years or less compared to healthy controls, and a less significant difference was found in patients with T2D for more than five years.

A recent study has reported that inhibition of the CXCL10/CXCR3 axis could enhance the β cell functionality and down-regulate the transcription of the pro-inflammatory mediators, nuclear factor κB (NF-κB) and STAT1, and therefore display an anti-inflammatory action in diabetes [16]. In concordance with this, another recent study has revealed that CXCL10 was increased in T2D patients, in comparison with healthy controls [17]. In our study, CXCL10 was observed at significantly higher levels in patients with T1D aged 5 years or less compared to other groups. Blocking CXCL10 at the time of diagnosis may be adopted as a new approach in the treatment of T1D.

In T1D and T2D, a complex inflammatory environment is developed in islet cells. In T1D, the secretion of anti-inflammatory cytokines (IL-4, IL-10, IL-13) is reduced, so their positive effects on beta cells are less [18]. Despite the low level of islet inflammation and lower levels of infiltrating immune cells in T2D compared to T1D, islet cells are still exposed to high levels of inflammatory mediators such as IL-6, TNF-alpha and IL-1beta [18]. Diminished levels of IL-10 in T2D patients were reported to be associated with insulin resistance [19].

In our study, patients with T1D for more than 5 years had higher IL-10 rates than those with T1D for 5 years or less. The IL-10 levels in the serum of patients who had T2D for less than five years were also found to be higher than the IL-10 levels of patients with T1D for five years or less. Exogenous administration of IL-13 was beneficial in the improvement of insulin sensitivity. On the other hand, IL-13 serum levels were demonstrated to be diminished in T2D patients [20]. While the presence of IL-13 in adipocytes was reported to be protective against insulin resistance [21]. Our study revealed a significant decrease in IL-13 levels in T1D and T2D patients for more than five years, in comparison with healthy subjects.

Conclusion

IL-10 rates in T1D patients remained low during the first five years and increased over longer periods. CXCL8 was higher among these patients, in comparison with patients with T2D. CXCL10 levels were also significantly higher in patients whose T1D was 5 years or less in comparison with the other groups. For IL-13, the results were in line with the literature. Although cytokines and chemokines were previously identified as immune molecules, they are now known to affect non-immune mechanisms as well. Therefore, it is beneficial to consider these factors almost equally in the diagnosis and treatment of both T1D and T2D. Future studies are required to warrant full illumination of the interrelations between diabetes pathogenesis and chemokines.

Limitations of the study

This study was designed to give an insight about the altered cytokine/chemokine levels in type1 as well as type2 diabetes patients, in comparison with that of healthy controls. This study design does not inform about the cellular sources of the cytokines/chemokines, which would be more informative. An analysis that is more detailed related with the disease durations and cytokine alterations, within the perspective of cellular sources would be more illuminative when the pathogenesis of the different diabetes types is taken into account.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: *This study was funded by the Scientific Research Projects Coordination Unit of Istanbul University. Project number: TYL-2017-23598*

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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How to cite this article:
Mina Ebrahimzadeh, Cemil Pehlivanoğlu, Burçin Aydın Özgür, Umut Can Küçüksezer, Sevgin Değirmencioğlu, Sakin Tekin, Ramazan Çakmak, Ahmet Dirican, Ali Osman Gürol. *IL-10, IL-13, IL-17 and CXCL8, CXCL10: Their relationship with the development and prognosis of type 1 and type 2 diabetes. Ann Clin Anal Med 2023;14(6):541-545*

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